



Macrofungal Survey of the Tian Shan Mountains, Kyrgyzstan

Sung Eun Cho, Jong Won Jo, Nam Kyu Kim, Young-Nam Kwag, Sang-Kuk Han, Kae Sun Chang, Seung Hwan Oh and Chang Sun Kim

Forest Biodiversity Division, Korea National Arboretum, Pocheon, Republic of Korea

ABSTRACT

The Tian Shan mountain system is one of the large mountain ranges located in Central Asia. This region is globally recognized as mountain ranges, offering inestimable wealth in fauna and flora with significant biodiversity values. We surveyed macrofungal diversity of Tian Shan in Kyrgyzstan from 2016 to 2018. A collection of macrofungi was made, and these were subjected to sequence comparisons and phylogenetic analysis to ensure the identity of the collected macrofungi. Of those collected, 95 out of 100 specimens were successfully sequenced and compared with those of other related species retrieved from GenBank. The sequenced specimens were classified into 2 phyla, 8 orders, 24 families, 47 genera, and 57 species, based on current taxonomic concepts (combining morphology and phylogeny). To the best of our knowledge, this study provides the first well-documented checklist and phylogenetic analysis of macrofungi recovered from the Tian Shan mountains in Kyrgyzstan.

ARTICLE HISTORY

Received 4 April 2019

Revised 5 June 2019

Accepted 24 August 2019

KEYWORDS

Ascomycota; basidiomycota; macrofungi; phylogeny; taxonomy

1. Introduction

Central Asia (Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, and Uzbekistan) represents ecologically most diverse regions, including steppes, tugai, tagai, wetlands, and deserts, thus biodiversity value is very high. The Tian Shan mountain system, known as mountains of heaven, is one of the large mountain ranges located in Central Asia. The mountainous territory is situated on the joint area of three states: Uzbekistan, Kazakhstan, and Kyrgyz Republic. The Tian Shan is commonly subdivided into four geographic sub-regions, consisting of the West, Inner, Northern, and Central Tian Shan regions [1]. In Tian Shan, over 80% of the countryside comprises valleys and basins, and the climate varies from dry continental to polar, depending on elevation.

The Tian Shan mountain range is the starting point of the Sino-Japanese floristic region, which includes the Korean peninsula, and is a region with a strong relationship with the native vegetation, geographical distribution, and phylogenetics of the Korea. However, biodiversity is threatened by the damage of the breeding ground resulting from the climate change, pasturing, and development. In this regard, the Korea National Arboretum (KNA) conducted a joint survey of forest biodiversity core areas in order to secure resources and establish biodiversity as well as basic data through research in Korea and Central Asia. In this study, we surveyed

macrofungal diversity in Tian Shan, Kyrgyzstan, from 2016 to 2018. This is the first paper ever published on the macrofungal diversity of Tian Shan area based on morphological observation and phylogenetic analysis.

2. Materials and methods

2.1. Macrofungi collection

During a field foray conducted from 2016 to 2018, the macrofungi were collected from the Inner Tian Shan in Kyrgyzstan (Figure 1). The specimens ($n=95$) are listed in Table 1, with identification information including GenBank accession numbers and similarity (%). The accession numbers of reliable internal transcribed spacer (ITS) sequences made in this study are provided and taxa are listed in alphabetical order. The systematics of the taxa applied in this study is in accordance with Index Fungorum (<http://www.indexfungorum.org>). Dried specimens studies were deposited in the herbarium of Korea National Arboretum (KNA).

2.2. Phylogenetic analysis

For the phylogenetic analysis, genomic DNAs from specimens were extracted using the DNeasy Plant Mini DNA Extraction Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instruction. The ITS

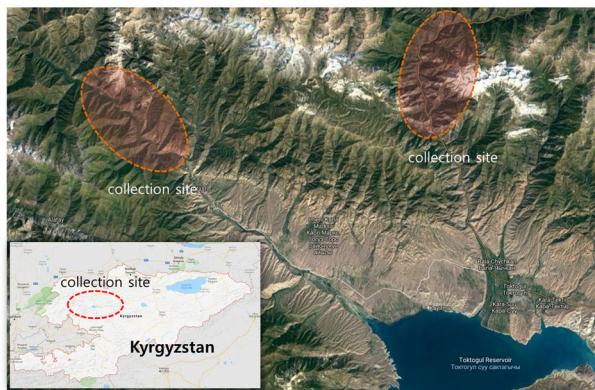


Figure 1. Map above is the regions of collection sites in Kyrgyzstan.

of rRNA regions were amplified with primers ITS5 and ITS4 as described [2]. The PCR amplicons were purified using a QIAquick purification kit (Qiagen Inc.) and directly sequenced using an ABI Prism™ 377 Automatic DNA Sequencer (Applied Biosystems, Foster City, CA) with a BigDye™ Cycle Sequencing Kit version 3.1 (Applied Biosystems). A Blastn search against the National Center for Biotechnology Information (NCBI) database (<http://www.ncbi.nlm.nih.gov>) was carried out, and the sequences were incorporated with those of the closely related species retrieved from public database (NCBI), which include Agaricomycetes, Sordariomycetes, and Pezizomycetes. The data set was then aligned using MAFFT version 7 (<https://mafft.cbrc.jp/alignment/server/>) [3]. A phylogenetic tree was constructed using RAxML in the CIPRES Science Gateway (<https://www.phylo.org>). *Entorrhiza aschersoniana* (KM359776) was used as an outgroup species. The relative robustness of the individual branches was estimated by bootstrapping using 1000 replicates.

2.3. Morphological observation

A total of 95 specimens were subjected to the identification based on the macroscopic and microscopic characteristics (Table 1). Dried materials mounted in distilled water, 5% KOH, 1% phloxine, Melzer's reagent and Congo red using a model of Olympus BX53 microscope and Jenoptik ProgRes C14 Plus Camera (Jenoptik Corporation, Jena, Germany). Microscopic parameters were measured using ProgRes Capture Pro software version 2.8.8. (Jenoptik Corporation).

3. Results

3.1. Molecular phylogenetic analysis

The PCR amplification and sequencing of ITS regions were successfully made for the specimens collected in this study. The resulting sequences were submitted to

GenBank, and the accession numbers were assigned (Acc. Nos. MK351663–MK351757). In addition, the identity of the species collected, accession numbers, closest match, accession numbers, and similarity were provided (Table 1). Although the similarity for some of the species identified is lower than 99%, when blasted against the NCBI database, the phylogenetic placement for the species was strongly supported with high bootstrap values (>90%), confirming its identity (e.g., *Coprinellus radians*, *Cyathus hookery*, *Cystoderma arcticum*, *Galerina discreta*, *Inocybe geophylla*, *Oxyporus cuneatus*, *Phellinus nigricans*, and *Protostropharia ovalispora*). However, the identity for 10 specimens, which include *Scutellinia* spp., *Inocybe* sp., *Mycena* sp., *Gymnopilus* sp., *Porodaedalea* sp., *Trichaptum* sp., and *Hygrocybe* sp., could not be ensured at the species level, since either no sequences from the NCBI database were available or no matches were achieved for the species when blasted against the NCBI database (Figure 2).

3.2. Macrofungal diversity

Based on our comprehensive phylogenetic and morphological analysis, the specimens were classified into 57 species, 47 genera, 24 families, and 8 orders in Ascomycota and Basidiomycota. Dominant species belonged to Polyporaceae (8 species), Agaricaceae (6 species), Hymenogastraceae, and Psathyrellaceae (4 species, respectively). Ten specimens could not be identified to the species level, due to insufficient information from the reference sequences and our specimens. Of those, it was confirmed as either saprophyte for 47 species or symbionts for 10 species (Figures 3–5). Some species identified are previously known from Kyrgyzstan; they are symbionts of trees and shrubs [4–7]. Our morphological descriptions and molecular sequence analysis provide the first large-scale macrofungal diversity assessment for the Tian Shan regions. Most of the macrofungi collected are of ecologically and economically importance; further, via their mycorrhizal associations, they play a central role in maintaining the health of the forest ecosystem.

3.3 Collected list of macrofungi from Tian Shan in this study

1. *Apioperdon pyriforme* (Schaeff.) Vizzini, in Vizzini & Ercole, Phytotaxa 299(1): 81. 2017.
Basionym: *Lycoperdon pyriforme* Schaeff. 1774
Specimen examined: August 10 2017, KA17-0639.
2. *Bovista plumbea* Pers., Ann. Bot. (Usteri) 15: 4. 1795.
Specimen examined: September 16 2016, KA16-1056.
3. *Coprinellus radians* (Fr.) Vilgalys, Hopple & Jacq. Johnson, in Redhead, Vilgalys, Moncalvo, Johnson & Hopple, Taxon 50(1): 234. 2001.

Table 1. List of collected macrofungi from Tian Shan in Kyrgyzstan.

No.	Scientific name	Specimen no.	GenBank accession no. (ITS)	Closest matched accession no.	Similarity (%)
1	<i>Jackrogersella multiformis</i>	KA16-1025	MK351664	KX394789	99
2	<i>Nectria dematiosa</i>	KA17-0619	MK351706	MH864605	99
3	<i>Pachyella</i> sp.	KA17-0624	MK351711	MH930382	85
4	<i>Peziza varia</i>	KA16-1061	MK351699	AF491559	99
5	<i>Peziza varia</i>	KA17-0622	MK351709	AF491559	99
6	<i>Scutellinia</i> sp. 1	KA16-1054	MK351693	KU556557	88
7	<i>Scutellinia</i> sp. 2	KA17-0623	MK351710	MF230412	99
8	<i>Scutellinia</i> sp. 3	KA18-0761	MK351733	MH930244	87
9	<i>Apioperdon pyriforme</i>	KA17-0639	MK351721	MF161171	100
10	<i>Bovista plumbea</i>	KA16-1056	MK351694	AJ237629	100
11	<i>Coprinellus radians</i>	KA18-0783	MK351750	JN943118	97
12	<i>Cortinarius imbutus</i>	KA18-0785	MK351752	KX964507	100
13	<i>Cortinarius ophiopus</i>	KA16-1027	MK351666	KF732609	99
14	<i>Crucibulum laeve</i>	KA16-1053	MK351692	MH855871	99
15	<i>Cyathus hookeri</i>	KA16-1024	MK351663	DQ463346	97
16	<i>Cystoderma arcticum</i>	KA16-1045	MK351684	GU234154	98
17	<i>Entoloma turci</i>	KA18-0790	MK351757	JF907993	99
18	<i>Fomes fomentarius</i>	KA17-0635	MK351718	JX910366	100
19	<i>Fomes fomentarius</i>	KA18-0774	MK351744	JX910366	100
20	<i>Fomitopsis pinicola</i>	KA16-1035	MK351674	MH931272	100
21	<i>Galerina discreta</i>	KA16-1042	MK351681	KR060631	98
22	<i>Ganoderma applanatum</i>	KA16-1032	MK351671	JX501311	100
23	<i>Ganoderma applanatum</i>	KA16-1033	MK351672	JX501311	100
24	<i>Ganoderma applanatum</i>	KA16-1038	MK351677	JX501311	100
25	<i>Ganoderma applanatum</i>	KA18-0755	MK351727	JX501311	100
26	<i>Ganoderma applanatum</i>	KA18-0775	MK351745	JX501311	100
27	<i>Gymnopilus spectabilis</i>	KA17-0628	MK351713	KT368688	99
28	<i>Gymnopilus</i> sp.	KA16-1048	MK351687	MH856206	99
29	<i>Hebeloma pseudofragilipes</i>	KA18-0756	MK351728	KX6872200	100
30	<i>Heterobasidion parviporum</i>	KA18-0753	MK351725	KC492952	99
31	<i>Heterobasidion parviporum</i>	KA18-0762	MK351734	KU645329	99
32	<i>Homophron cernuum</i>	KA16-1065	MK351703	AM712286	99
33	<i>Hygrocybe</i> sp.	KA18-0757	MK351729	FR750604	98
34	<i>Inocybe geophylla</i>	KA18-0789	MK351756	KY990539	89
35	<i>Inocybe</i> sp.	KA18-0787	MK351754	JX630727	99
36	<i>Leccinum scabrum</i>	KA18-0772	MK351743	KC552012	99
37	<i>Leccinum scabrum</i>	KA18-0786	MK351753	KC552012	99
38	<i>Lentinus brumalis</i>	KA16-1044	MK351683	KX851607	99
39	<i>Lentinus brumalis</i>	KA17-0630	MK351715	KX851607	99
40	<i>Lentinus brumalis</i>	KA18-0758	MK351730	KX851607	99
41	<i>Lentinus brumalis</i>	KA18-0781	MK351749	KX851607	99
42	<i>Lycoperdon frigidum</i>	KA17-0629	MK351714	DQ112568	100
43	<i>Melanoleuca cognata</i>	KA16-1046	MK351685	JX429225	100
44	<i>Melanoleuca cognata</i>	KA16-1047	MK351686	JX429225	100
45	<i>Mycena galericulata</i>	KA16-1031	MK351670	KJ705178	99
46	<i>Mycena galericulata</i>	KA16-1062	MK351700	KJ705178	99
47	<i>Mycena</i> sp.	KA18-0760	MK351732	JF519505	100
48	<i>Mycenastrum corium</i>	KA17-0641	MK351723	EU833666	99
49	<i>Oxyporus cuneatus</i>	KA18-0779	MK351747	DQ384575	94
50	<i>Panaeolus rickenii</i>	KA16-1041	MK351680	KY559329	99
51	<i>Parasola plicatilis-similis</i>	KA16-1040	MK351679	KY928621	99
52	<i>Parmastomyces corticola</i>	KA18-0784	MK351751	HQ848472	99
53	<i>Phellinus laevigatus</i>	KA16-1026	MK351665	GQ383779	100
54	<i>Phellinus nigricans</i>	KA16-1028	MK351667	MF319077	99
55	<i>Phellinus nigricans</i>	KA17-0621	MK351708	MF319077	98
56	<i>Phellinus nigricans</i>	KA18-0752	MK351724	MF319077	99
57	<i>Phellinus nigricans</i>	KA18-0765	MK351737	MF319077	98
58	<i>Phellinus nigricans</i>	KA16-1060	MK351698	KU139172	99
59	<i>Phlebia tremellosa</i>	KA16-1052	MK351691	MH857589	99
60	<i>Phlebia tremellosa</i>	KA18-0766	MK351738	MK172823	100
61	<i>Phlebia tremellosa</i>	KA18-0777	MK351746	MF303710	100
62	<i>Pholiota mutabilis</i>	KA16-1057	MK351695	KX449454	99
63	<i>Pholiota mutabilis</i>	KA18-0759	MK351731	KX449454	99
64	<i>Pholiota mutabilis</i>	KA18-0780	MK351748	KX449454	99
65	<i>Pholiota squarrosa</i>	KA16-1037	MK351676	KM044075	100
66	<i>Pholiota squarrosa</i>	KA16-1049	MK351688	FR686575	100
67	<i>Pholiota squarrosa</i>	KA16-1064	MK351702	FR686575	99
68	<i>Pholiota squarrosa</i>	KA18-0768	MK351739	FR686575	99
69	<i>Picipes badius</i>	KA16-1063	MK351701	MK404672	99
70	<i>Porodaedalea</i> sp.	KA18-0769	MK351740	JX110044	99
71	<i>Porostereum spadiceum</i>	KA17-0636	MK351719	MH856439	99
72	<i>Protostropharia ovalispora</i>	KA16-1039	MK351678	KR998381	96
73	<i>Psathyrella warrenensis</i>	KA16-1043	MK351682	KC992906	99
74	<i>Pycnoporus cinnabarinus</i>	KA16-1036	MK351675	AF363757	99
75	<i>Pycnoporus cinnabarinus</i>	KA17-0634	MK351717	AF313765	99
76	<i>Russula atroglauca</i>	KA18-0763	MK351735	MG367260	99

(continued)

Table 1. Continued.

No.	Scientific name	Specimen no.	GenBank accession no. (ITS)	Closest matched accession no.	Similarity (%)
77	<i>Schizophyllum commune</i>	KA17-0625	MK351712	MK256472	100
78	<i>Schizophyllum commune</i>	KA17-0633	MK351716	MK256472	100
79	<i>Schizophyllum commune</i>	KA18-0764	MK351736	MH301329	100
80	<i>Stereum hirsutum</i>	KA16-1058	MK351696	AY854063	100
81	<i>Stereum hirsutum</i>	KA17-0620	MK351707	AY854063	100
82	<i>Trametes hirsuta</i>	KA16-1029	MK351668	MH860685	99
83	<i>Trametes hirsuta</i>	KA16-1030	MK351669	MK838877	100
84	<i>Trametes hirsuta</i>	KA16-1051	MK351690	MH860685	100
85	<i>Trametes hirsuta</i>	KA16-1067	MK351705	MH860685	99
86	<i>Trametes hirsuta</i>	KA18-0771	MK351742	MK838877	99
87	<i>Trametes hirsuta</i>	KA18-0788	MK351755	MH860685	100
88	<i>Trametes ochracea</i>	KA16-1059	MK351697	MH855443	99
89	<i>Trametes ochracea</i>	KA17-0638	MK351720	MH248060	99
90	<i>Trametes ochracea</i>	KA17-0640	MK351722	MH855443	100
91	<i>Trametes ochracea</i>	KA18-0754	MK351726	MH855443	100
92	<i>Trametes ochracea</i>	KA18-0770	MK351741	MH855443	100
93	<i>Trametes versicolor</i>	KA16-1034	MK351673	MH855443	100
94	<i>Trametes versicolor</i>	KA16-1066	MK351704	MK256462	100
95	<i>Trichaptum</i> sp.	KA16-1050	MK351689	MH114914	97

Basic nomenclatural data are cited according to Index Fungorum. Specimens used in the molecular phylogenetic study with GenBank accession numbers are given.

- Basionym:** *Coprinus radians* Fr. 1838.
 Specimen examined: August 30 2018,
 KA18-0783.
4. ***Cortinarius imbutus*** Fr., Epicr. syst. mycol. (Upsaliae): 306. 1838.
 Specimen examined: August 30 2018,
 KA18-0785.
5. ***Cortinarius ophiopus*** Peck, Ann. Rep. N.Y. St. Mus. nat. Hist. 30: 43. 1878 [1877]
 Specimen examined: September 14 2016,
 KA16-1027.
6. ***Crucibulum laeve*** (Huds.) Kambly, Gast. Iowa: 167. 1936.
Basionym: *Peziza laevis* Huds. 1778.
 Specimen examined: September 15 2016,
 KA16-1053.
7. ***Cyathus hookeri*** Berk., Hooker's J. Bot. Kew Gard. Misc. 6: 204. 1854.
 Specimen examined: September 14 2016,
 KA16-1024.
8. ***Cystoderma arcticum*** Harmaja, Karstenia 24(1): 31. 1984.
 Specimen examined: September 15 2016,
 KA16-1045
9. ***Entoloma turci*** (Bres.) M. M. Moser, in Gams, Kl. Krypt.-Fl., Bd II b/2, ed. 4 (Stuttgart) 2b/2: 200. 1978.
Basionym: *Leptonia turci* Bres. 1884.
 Specimen examined: August 30 2018,
 KA18-0790.
10. ***Fomes fomentarius*** (L.) Fr., Summa veg. Scand., Sectio Post. (Stockholm): 321. 1849.
Basionym: *Boletus fomentarius* L. 1753.
 Specimens examined: August 10 2017, KA17-0635; 29 Aug. 2018, KA18-0774.
11. ***Fomitopsis pinicola*** (Sw.) P. Karst., Meddn Soc. Fauna Flora fenn. 6: 9. 1881.
- Basionym:** *Boletus pinicola* Sw. 1810.
 Specimen examined: September 14 2016,
 KA16-1035.
12. ***Galerina discreta*** E. Horak, Senn-Irlet, Curti & Musumeci, Riv. Mic. 52(2): 99. 2009.
 Specimen examined: September 15 2016,
 KA16-1042.
13. ***Ganoderma applanatum*** (Pers.) Pat., Hyménomyc. Eur. (Paris): 143. 1887.
Basionym: *Boletus applanatus* Pers. 1800.
 Specimens examined: September 14 2016, KA16-1032; September 14 2016, KA16-1033; September 14 2016, KA16-1038; August 28 2018, August 28 2018, KA18-0755; August 29 2018, KA18-0775.
14. ***Gymnopilus spectabilis*** (Fr.) Singer, November Holland. pl. spec.: 471. 1951.
Basionym: *Agaricus junonius* Fr. 1821
 Specimen examined: August 9 2017, KA17-0628.
15. ***Gymnopilus* sp.**
 Specimen examined: September 15 2016,
 KA16-1048.
 Note: Rees et al. [8] found ITS sequence variation in genus *Gymnopilus*. Our phylogenetic tree shows that this specimen is closely related to *Gymnopilus sapineus* and *Gymnopilus penetrans*. However, the basidiospore size of this collection (11.7–20 × 10.3–11.7 µm) differs from those of *G. sapineus* (7.2–9.2 × 4.5–5.2 µm) [9] and *G. penetrans* (syn. *G. hybridus*, 7.2–8.8 × 4.4–5.2 µm) [9]. Thus, this collection has larger and wider basidiospores than collections previously reported [9]. Here, we tentatively consider this collection to be *Gymnopilus* sp.; further sampling is required to verify this specimen (Figures 3–5).
16. ***Hebeloma pseudofragilipes*** Beker, Vesterh. & U. Eberh., in Beker, Eberhardt, Vesterholt & Schütz, Fungal Biology 120(1): 88. 2016.

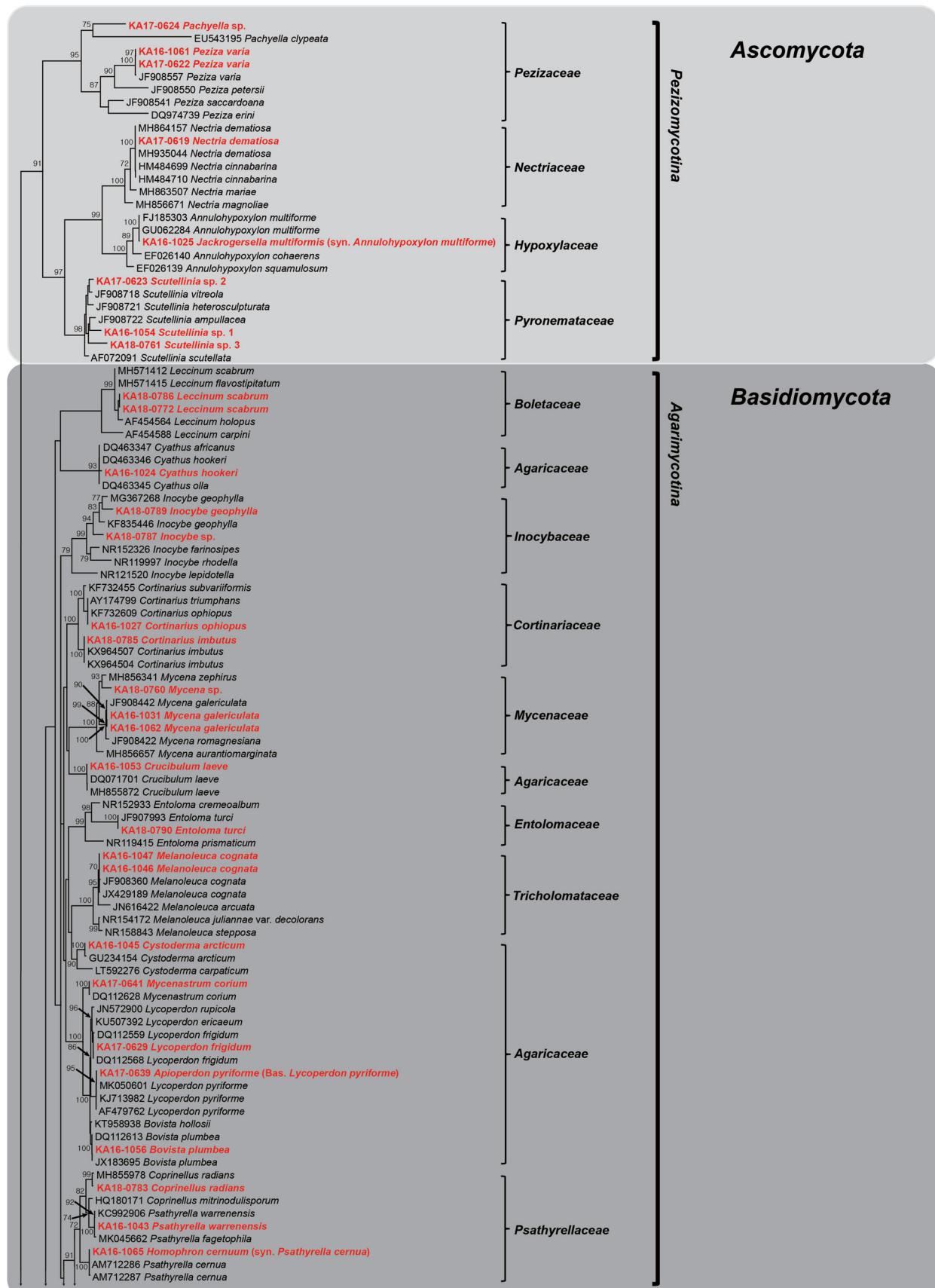


Figure 2. RAxML tree of macrofungal species using internal transcribed spacer (ITS) regions sequences. Bootstrapping values higher than 70% are shown in the branches (1000 replicates). The scale bar equals the number of nucleotide substitutions per site (continued).

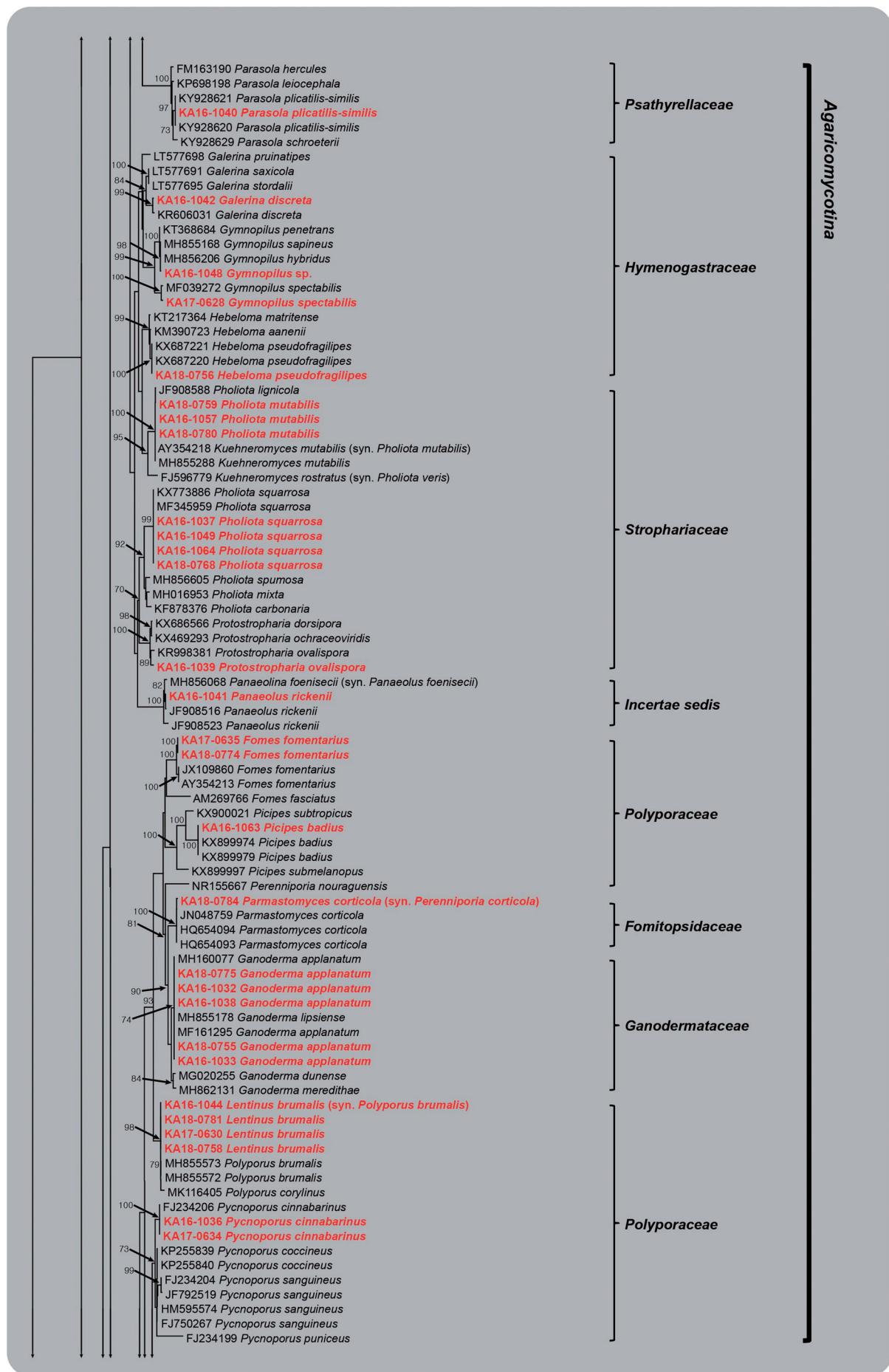


Figure 2. Continued

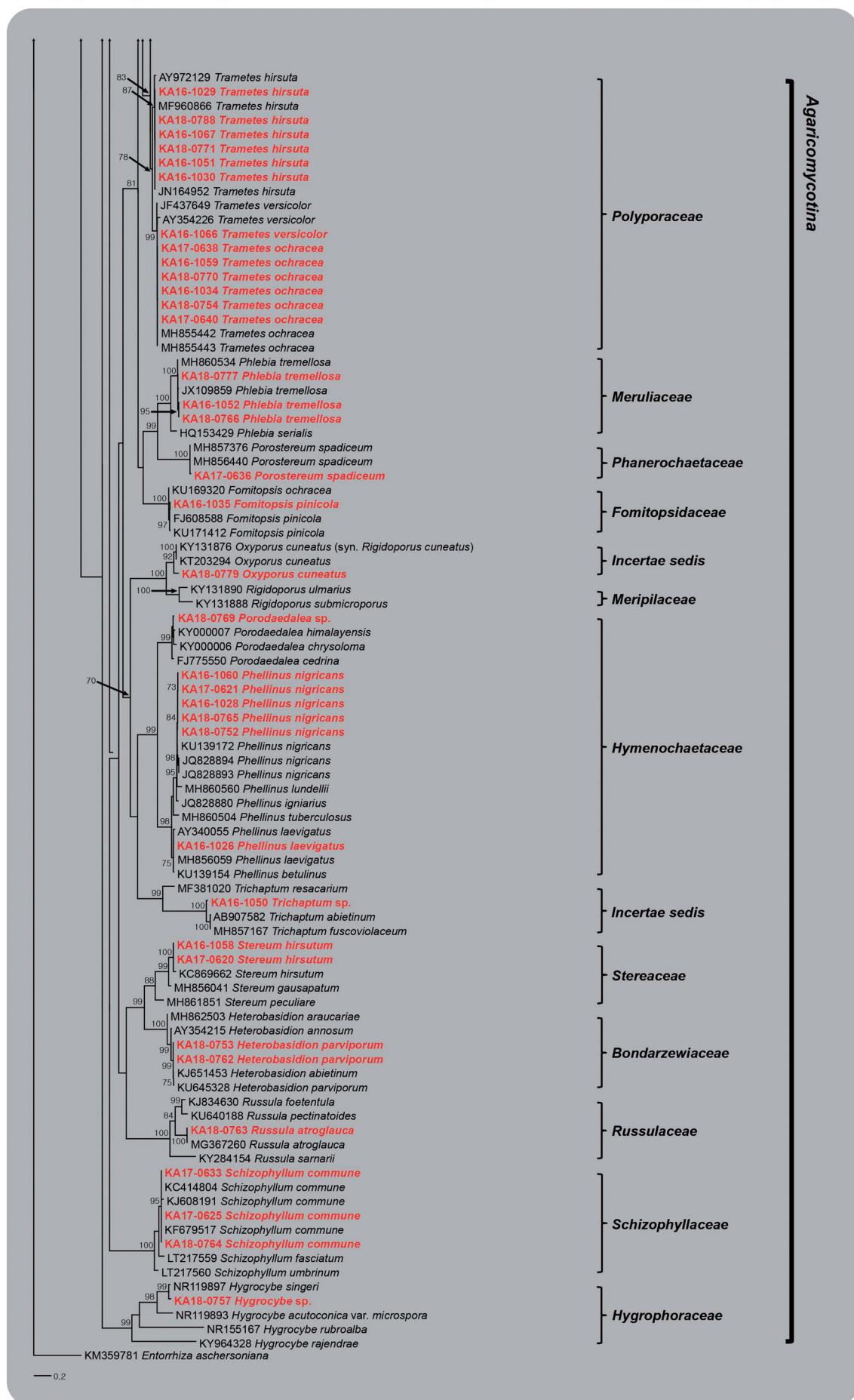


Figure 2. Continued



Figure 3. Morphological diversity of macrofungi from Tian Shan in Kyrgyzstan. (1) *Apioperdon pyriforme* (KA17-0639); (2) *Bovista plumbea* (KA16-1056); (3) *Coprinellus radians* (KA18-0783); (4) *Cortinarius imbutus* (KA18-0785); (5) *Cortinarius ophiopus* (KA16-1027); (6) *Crucibulum laeve* (KA16-1053); (7) *Cyathus hookeri* (KA16-1024); (8) *Cystoderma arcticum* (KA16-1045); (9) *Entoloma turci* (KA18-0790); (10) *Fomes fomentarius* (KA18-0774); (11) *Fomitopsis pinicola* (KA16-1035); (12) *Galerina discreta* (KA16-1042); (13) *Ganoderma applanatum* (KA18-0775); (14) *Gymnopilus spectabilis* (KA17-0628); (15) *Gymnopilus* sp. (KA16-1048); (16) *Hebeloma pseudofragilipes* (KA18-0756); (17) *Heterobasidion parviporum* (KA18-0762); (18) *Homophron cernuum* (KA16-1065); (19) *Hygrocybe* sp. (KA18-0757); (20) *Inocybe geophylla* (KA18-0789). Scale bars = 3 cm.

Specimen examined: August 28 2018,
KA18-0756.

17. *Heterobasidion parviporum* Niemelä & Korhonen, *Heterobasidion annosum*, Biology, Ecology, Impact and Control (Wallingford): 31. 1998.
Specimens examined: August 28 2018, KA18-0753; 28 August 28 2018, KA18-0762.

18. *Homophron cernuum* (Vahl) Örstadius & E. Larss., in Örstadius, Ryberg & Larsson, Mycol. Progr. 14(25): 36. 2015.

Basionym: *Agaricus cernuus* Vahl 1790.

Specimen examined: September 16 2016,
KA16-1065.

19. *Hygrocybe* sp.



Figure 4. (21) *Inocybe* sp. (KA18-0787); (22) *Jackrogersella multiformis* (KA16-1025); (23) *Leccinum scabrum* (KA18-0786); (24) *Lentinus brumalis* (KA16-1044); (25) *Lycoperdon frigidum* (KA17-0629); (26) *Melanoleuca cognata* (KA16-1047); (27) *Mycena galericulata* (KA16-1031); (28) *Mycena* sp. (KA18-0760); (29) *Mycenastrum corium* (KA17-0641); (30) *Nectria dematiosa* (KA17-0619); (31) *Oxyporus cuneatus* (KA18-0779); (32) *Pachyella* sp. (KA17-0624); (33) *Panaeolus rickenii* (KA16-1041); (34) *Parasola plicatilis-similis* (KA16-1040); (35) *Parmastomyces corticola* (KA18-0784); (36) *Peziza varia* (KA17-0622); (37) *Phellinus laevigatus* (KA16-1026); (38) *Phellinus nigricans* (KA18-0765); (39) *Phlebia tremellosa* (KA18-0777); (40) *Pholiota mutabilis* (KA18-0780). Scale bars = 3 cm.

Specimen examined: August 28 2018, KA18-0757.

Note: According to Lodge et al. [10], genus *Hygrocybe* is paraphyletic within the Hygrophoraceae. Our phylogenetic analysis reveals that this specimen is closely related to *Hygrocybe singeri*. However, the basidiospore size of this specimen ($5.9\text{--}8.8 \times 4.4\text{--}5.8 \mu\text{m}$) is

different from that of *H. singeri* ($9.0\text{--}12 \times 5.0\text{--}6.0 \mu\text{m}$) [11]. Therefore, we treat this specimen as *Hygrocybe* sp.

20. *Inocybe geophylla* (Bull.) P. Kumm., Führ. Pilzk. (Zerbst): 78. 1871.

Basionym: *Agaricus geophyllus* Bull. 1791.

Specimen examined: August 30 2018, KA18-0789.

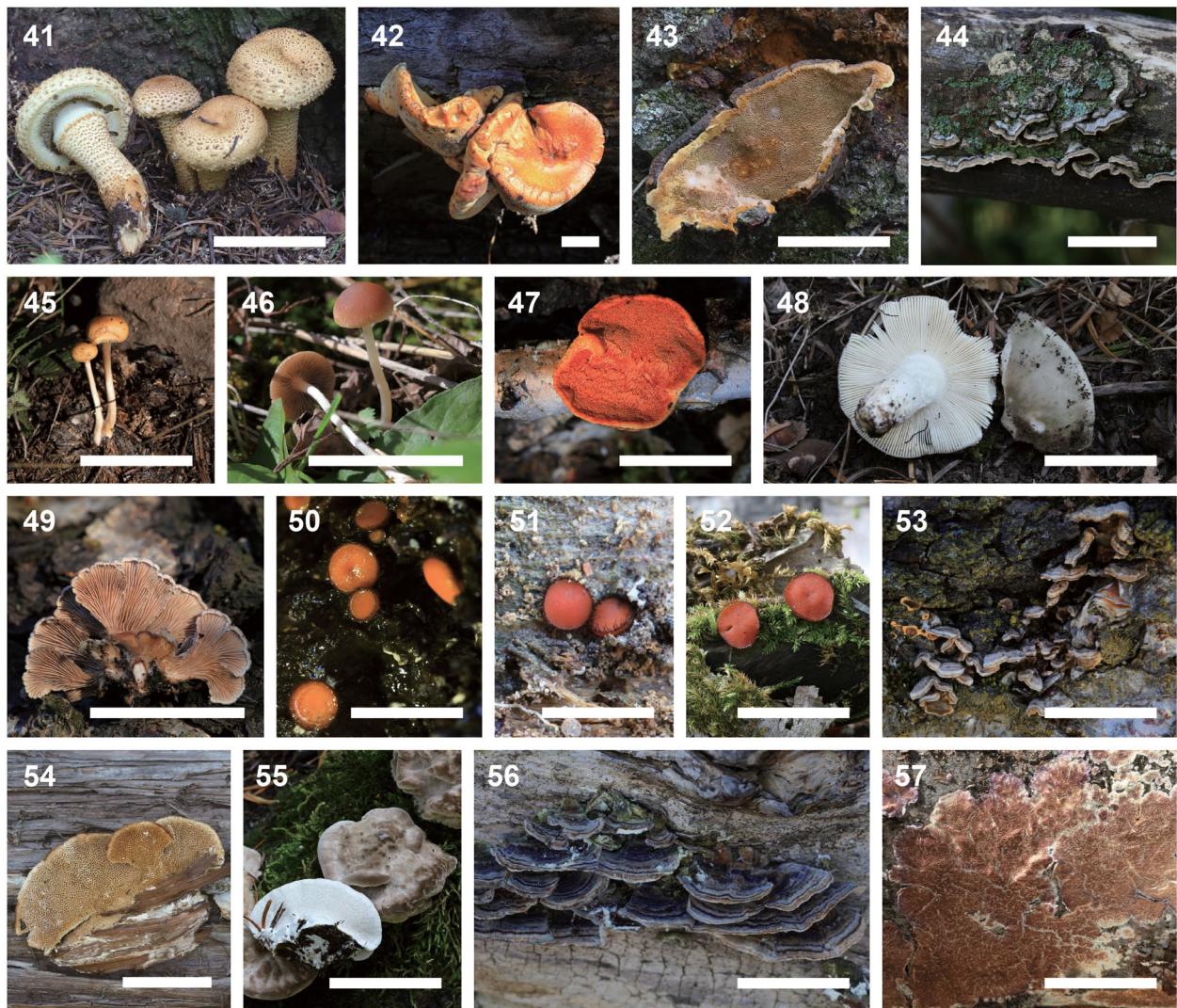


Figure 5. (41) *Pholiota squarrosa* (KA18-0768); (42) *Picipes badius* (KA16-1063); (43) *Porodaedalea* sp. (KA18-0769); (44) *Porostereum spadiceum* (KA17-0636); (45) *Protostropharia ovalispora* (KA16-1039); (46) *Psathyrella warrenensis* (KA16-1043); (47) *Pycnoporus cinnabarinus* (KA17-0634); (48) *Russula atroglauca* (KA18-0763); (49) *Schizophyllum commune* (KA17-0633); (50) *Scutellinia* sp. 1 (KA16-1054); (51) *Scutellinia* sp. 2 (KA17-0623); (52) *Scutellinia* sp. 3 (KA18-0761); (53) *Stereum hirsutum* (KA16-1058); (54) *Trametes hirsuta* (KA18-0788); (55) *Trametes ochracea* (KA18-0770); (56) *Trametes versicolor* (KA16-1066); (57) *Trichaptum* sp. (KA16-1050). Scale bars = 3 cm.

21. *Inocybe* sp.

Specimen examined: August 30 2018, KA18-0787.

Note: We could not compare this collection with reference sequence database at NCBI. Because it is difficult to identify to species level from one specimen, further sampling and analysis is required for this species.

22. *Jackrogersella multiformis* (Fr.) L. Wendt, Kuhnert & M. Stadler, in Wendt, Sir, Kuhnert, Heitkämper, Lambert, Hladki, Romero, Luangsaard, Srikitkulchai, Persoh & Stadler, Mycol. Progr.: 10.1007/s11557-017-1311-3, 2017.

Basionym: *Sphaeria multiformis* Fr. 1815.

Specimen examined: September 14 2016, KA16-1025.

23. *Leccinum scabrum* (Bull.) Gray, Nat. Arr. Brit. Pl. (London) 1: 647.1821.

Basionym: *Boletus scaber* Bull. 1783.

Specimens examined: August 28 2018, KA18-0772; August 30 2018, KA18-0786.

24. *Lentinus brumalis* (Pers.) Zmitr., International Journal of Medicinal Mushrooms (Redding) 12(1): 88. 2010.

Basionym: *Boletus brumalis* Pers. 1794.

Specimens examined: September 15 2016, KA16-1044; August 10 2017, KA17-0630; August 28 2018, KA18-0758; August 30 2018, KA18-0781.

25. *Lycoperdon frigidum* Demoulin, Lejeunia 62: 10. 1972.

Specimen examined: August 10 2017, KA17-0629.

26. *Melanoleuca cognata* (Fr.) Konrad & Maubl., Icon. Select. Fung. 3(2): pl. 271. 1927.

Basionym: *Agaricus arcuatus* var. *cognatus* Fr. 1874.

Specimens examined: September 15 2016, KA16-1046, KA16-1047.

27. *Mycena galericulata* (Scop.) Gray, Nat. Arr. Brit. Pl. (London) 1: 619. 1821.
Basionym: *Agaricus galericulatus* Scop. 1772.
 Specimens examined: September 14 2016, KA16-1031; September 16 2016, KA16-1062.
28. *Mycena* sp.
 Specimen examined: August 28 2018, KA18-0760.
 Note: Although this specimen is phylogenetically close to *Mycena zephyrus*, it differs morphologically from *M. zephyrus*. The size of basidiospores of this collection ($5.8\text{--}8.8 \times 3.0\text{--}4.4 \mu\text{m}$) is smaller than those of *M. zephyrus* ($8.5\text{--}9.3\text{--}10.4 \times 5.3\text{--}5.9\text{--}6.6 \mu\text{m}$) [12]. Thus, additional specimens are needed to identify.
29. *Mycenastrum corium* (Guers.) Desv., Annls Sci. Nat., Bot., sér. 2 17: 147. 1842.
Basionym: *Lycoperdon corium* Guers., in Lamarck & de Candolle 1805.
 Specimen examined: August 10 2017, KA17-0641.
30. *Nectria dematiosa* (Schwein.) Berk., N. Amer. Fung.: no. 154. 1873.
Basionym: *Sphaeria dematiosa* Schwein. 1832.
 Specimen examined: August 9 2017, KA17-0619.
31. *Oxyporus cuneatus* (Murrill) Aoshima, Trans. Mycol. Soc. Japan 8(1): 3. 1967.
Basionym: *Coriolellus cuneatus* Murrill 1907.
 Specimen examined: August 29 2018, KA18-0779.
32. *Pachyella* sp.
 Specimen examined: August 9 2017, KA17-0624.
 Note: This specimen is verified as *Pachyella*, based on macroscopic characteristics. Because there are few ITS sequences of *Pachyella* species in GenBank, phylogenetic comparison of *Pachyella* species is difficult. Our specimen is positioned on a long branch, indicating that it is phylogenetically different from the other *Pachyella* species in this study. This species is thus tentatively maintained as *Pachyella* sp.
33. *Panaeolus rickenii* Hora, Trans. Br. mycol. Soc. 43(2): 454. 1960.
 Specimen examined: September 15 2016, KA16-1041.
34. *Parasola plicatilis-similis* L. Nagy, Szarkándi & Dima, in Szarkándi, Schmidt-Stohn, Dima, Hussain, Kocsubé, Papp, Vágvölgyi & Nagy, Mycologia 109(4): 626. 2017.
 Specimen examined: September 15 2016, KA16-1040.
35. *Parmastomyces corticola* Corner [as "corticicola"], Beih. Nova Hedwigia 96: 96. 1989.
Synonym: *Perenniporia corticola* (Corner) Decock, Mycologia 93(4): 776. 2001.
 Specimen examined: August 30 2018, KA18-0784.
36. *Peziza varia* (Hedw.) Alb. & Schwein., Consp. fung. (Leipzig): 311. 1805.
Basionym: *Octospora varia* Hedw. 1789.
 Specimens examined: August 16 2016, KA16-1061; September 9 2017, KA17-0622.
37. *Phellinus laevigatus* (P. Karst.) Bourdot & Galzin, Hyménomyc. de France (Sceaux): 624. 1928. [1927].
Basionym: *Poria laevigata* P. Karst. 1881.
 Specimen examined: September 14 2016, KA16-1026.
38. *Phellinus nigricans* (Fr.) P. Karst., Finl. Basidsvamp. no. 11: 134. 1899.
Basionym: *Polyporus nigricans* Fr. 1821.
 Specimens examined: September 14 2016, KA16-1028; September 9 2017, KA17-0621; August 27 2018, KA18-0752; August 28 2018, KA18-0765; September 16 2016, KA16-1060.
39. *Phlebia tremellosa* (Schrad.) Nakasone & Burds. [as "tremellosum"], Mycotaxon 21: 245. 1984.
Basionym: *Merulius tremellosus* Schrad. 1794.
 Specimens examined: September 15 2016, KA16-1052; August 28 2018, KA18-0766; August 29 2018, KA18-0777.
40. *Pholiota mutabilis* (Schaeff.) P. Kumm., Führ. Pilzk. (Zerbst): 83. 1871.
Basionym: *Agaricus mutabilis* Schaeff. 1774.
 Specimens examined: September 16 2016, KA16-1057; August 28 2018, KA18-0759; August 30 2018, KA18-0780.
41. *Pholiota squarrosa* (Vahl) P. Kumm., Führ. Pilzk. (Zerbst): 83. 1871.
Basionym: *Agaricus squarrosus* Vahl, in Oeder 1770.
 Specimens examined: September 14 2016, KA16-1037; September 15 2016, KA16-1049; September 16 2016, KA16-1064; August 28 2018, KA18-0768.
42. *Picipes badius* (Pers.) Zmitr. & Kovalenko, International Journal of Medicinal Mushrooms (Redding) 18(1): 35. 2016.
Basionym: *Boletus badius* Pers. 1801.
 Specimen examined: September 16 2016, KA16-1063.
43. *Porodaedalea* sp.
 Specimen examined: August 28 2018, KA18-0769.
 Note: Based on basidiospore size, this specimen ($4.4\text{--}5.8 \times 4.4\text{--}5.9 \mu\text{m}$) is morphologically similar to *Porodaedalea himalaensis* ($4.3\text{--}5.8 \times 3.5\text{--}4.8 \mu\text{m}$) [13]. However, based on phylogenetic analysis, this specimen is closely related to *P. himalaensis* and *Porodaedalea chrysoloma*. Therefore, we tentatively assigned this specimen to *Porodaedalea* sp. Further sampling of *Porodaedalea* in Kyrgyzstan is required to resolve the identity of this specimen.

44. *Porostereum spadiceum* (Pers.) Hjortstam & Ryvarden, Syn. Fung. (Oslo) 4: 51. 1990.
Basionym: Thelephora spadicea Pers. 1801.
 Specimen examined: August 10 2017, KA17-0636.
45. *Protostropharia ovalispora* Yen W. Wang & S.S. Tzean, Taiwania 60(4): 162. 2015.
 Specimen examined: September 14 2016, KA16-1039.
46. *Psathyrella warrenensis* A.H. Sm., Contr. Univ. Mich. Herb. 5: 416. 1972.
 Specimen examined: September 15 2016, KA16-1043.
47. *Pycnoporus cinnabarinus* (Jacq.) P. Karst., Revue mycol., Toulouse 3(9): 18. 1881.
Basionym: Boletus cinnabarinus Jacq. 1776.
 Specimens examined: September 14 2016, KA16-1036; August 10 2017, KA17-0634.
48. *Russula atroglauca* Einhell., Denkschr. Regensb. bot. Ges. 39: 101. 1980.
 Specimen examined: August 28 2018, KA18-0763.
49. *Schizophyllum commune* Fr. [as "Schizophyllum communis"], Observ. mycol. (Havniae) 1: 103. 1815.
 Specimens examined: August 9 2017, KA17-0625; August 10 2017, KA17-0633; August 28 2018, KA18-0764.
50. 51, 52. ***Scutellinia* spp. 1, 2, and 3**
 Specimens examined: September 15 2016, KA16-1054; August 9 2017, KA17-0623; August 28 2018, KA18-0761.
 Note: These collections belong to *Scutellinia*, based on morphology and ITS sequence analysis. Morphologically, the basidiospore sizes of the three samples we collected ($14\text{--}18 \times 10\text{--}12 \mu\text{m}$) were almost indistinguishable. These specimens do not cluster together with other *Scutellinia* species in our phylogenetic tree. Therefore, more sampling, multi-locus phylogenetic analysis, and morphological observation will be required for accurate identification. Therefore, we tentatively designated these specimens *Scutellinia* spp. 1, 2, and 3.
53. *Stereum hirsutum* (Willd.) Pers., Observ. mycol. (Lipsiae) 2: 90. 1800.
Basionym: Thelephora hirsuta Willd. 1787.
 Specimens examined: September 16 2016, KA16-1058; August 9 2017, KA17-0620.
54. *Trametes hirsuta* (Wulfen) Lloyd, Mycol. Writ. 7(Letter 73): 1319. 1924.
Basionym: Boletus hirsutus Wulfen, in Jacquin 1791.
 Specimens examined: September 14 2016, KA16-1029, KA16-1030; September 15 2016, KA16-1051; September 16 2016, KA16-1067; August 28 2018, KA18-0771; August 30 2018, KA18-0788.
55. *Trametes ochracea* (Pers.) Gilb. & Ryvarden, N. Amer. Polyp., Vol. 2 Megasporoporia - Wrightoporia (Oslo): 752. 1987.
Basionym: Boletus ochraceus Pers. 1794.
 Specimens examined: September 14 2016, KA16-1034; September 16 2016, KA16-1059; August 10 2017, KA17-0638; August 10 2017, KA17-0640; August 8 2018, KA18-0754; August 28 2018, KA18-0770.
 Note: *Trametes ochacea* is closely related to *Trametes versicolor* in ITS tree (Figure 2). However, they could easily distinguish by the size of basidiospores (*T. ochacea*: $6.7 \times 2.2.5 \mu\text{m}$; *T. versicolor*: $4.5\text{--}5.5 \times 1.5\text{--}2 \mu\text{m}$).
56. *Trametes versicolor* (L.) Lloyd, Mycol. Notes (Cincinnati) 65: 1045. 1921.
Basionym: Boletus versicolor L. 1753.
 Specimen examined: September 16 2016, KA16-1066.
57. ***Trichaptum* sp.**
 Specimen examined: September 15 2016, KA16-1050.
 Note: Because we had too few specimens, we were unable to study this collection morphologically. This collection clusters in a highly supported *Trichaptum* clade, and is phylogenetically distinct from the closest related species, *Trichaptum abietinum* and *Trichaptum fuscoviolaceum*. Because it contains DNA sequence polymorphism, it is difficult to identify based on molecular sequence analysis [14]. Therefore, this collection is maintained as *Trichaptum* sp. until more information becomes available.

4. Discussion

The territory of Kyrgyzstan is renowned for its high biodiversity, with inestimable wealth in fauna and flora including animals, bacteria, fungi, plants, and viruses. Among these, 2,188 macrofungal species, including Myxomycetes (slime fungi), have been reported in Kyrgyzstan (CBD Fifth National Report – Kyrgyzstan 2013). In addition, the diversity of endophytic fungi from the wild medicinal plants of Northern and Northeastern Tian Shan was previously studied [15]. However, these reports just mention the checklist and we could not find the specimens that they deposited. In addition, little is known about the sequences data and morphological descriptions of previously recorded species in Kyrgyzstan.

According to Prikhodko et al. [16], they collected 43 species of macromycetes that belong to 17 families and 28 genera in Ala-Archa national park located in central portion of the northern

macroslope of Kyrgyz range of the Tian Shan mountain. Although our macrofungal collections are different from those made by Prikhodko et al. [16] where *Russula* species were mostly collected, but no species of *Russula* were found in this study. The finding for two species, *Bovista plumbea* and *Leccinum scabrum*, is consistent with our study. The Ala-Archa national park is located in the Tian Shan mountains of Kyrgyzstan, which is approximately 130 km away from the Tian Shan regions that we surveyed. In this regard, the distribution pattern for macrofungi, depending on the altitude, climate conditions, vegetation of mountain area, and temperature, might be differed significantly, resulting in substantial difference in the macrofungal diversity.

Previous studies for the macrofungi in Kyrgyzstan were only based on the simple morphological identification, providing the brief checklists. This is contrast to the study where 57 macrofungal species were identified from the Tian Shan mountain ranges of Kyrgyzstan based on the morphological characteristics and ITS sequence analysis. Given the fact that the fungal biodiversity from the areas is understudied, new species with unusual features remain to be uncovered. Therefore, future taxonomic studies based on molecular analysis with current taxonomic concepts are needed to better understand fungal diversity from the areas and accurately assure the fungal identity.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was supported by the research fund of Korea National Arboretum [Project Nos. KNA 1-1-17, 15-2; KNA 1-1-22, 17-2].

References

- [1] Farrington JD. A report on protected areas, biodiversity, and conservation in the Kyrgyzstan Tian Shan with brief notes on the Kyrgyzstan Pamir-Alai and the Tian Shan mountains of Kazakhstan, Uzbekistan, and China: U.S. Fulbright Program, Environmental Studies Section; 2005.
- [2] White TJ, Bruns T, Lee S, et al. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, editors. PCR protocols: a guide to methods and applications. New York (NY): Academic Press Inc.; 1990. p. 315–322.
- [3] Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013; 30(4):772–780.
- [4] Elchibaev AA. Macromycetes of the Northern Kyrgyzia. Ilim Frunze. 1968;93. (in Russian).
- [5] Pospelov AG, Zaprometov NG, Domashova AA. Fungal flora of the Kyrgyz SSR. Frunze Acad. Sci Kyrg SSR. 1957;1:129. (in Russian).
- [6] Prikhodko SL, Mosolova SN. Edible and poisonous mushrooms of Kyrgyzstan. Bishkek Ilim. 2000;50. (in Russian).
- [7] Prikhodko SL, Mosolova SN. Edible mushrooms of the Kyrgyzstan forests. In: Studies of the wildlife in Kyrgyzstan, Bishkek. 2002. p. 173–176. (In Russian).
- [8] Rees BJ, Marchant A, Zuccarello GC. A tale of two species – Possible origins of red to purple-coloured *Gymnopilus* species in Europe. Australas Mycol. 2004;22:57–72.
- [9] Holec J. The genus *Gymnopilus* (Fungi, Agaricales) in the Czech Republic with respect to collections from other European countries. Acta Musei Natl Pragae Ser B – Hist Nat. 2005;61:1–52.
- [10] Lodge DJ, Padamsee M, Matheny PB, et al. Molecular phylogeny, morphology, pigment chemistry and ecology in *Hygrophoraceae* (Agaricales). Fungal Divers. 2014;64(1):1–99.
- [11] Smith AH, Hesler LR. Additional North American Hygrophori. Sydowia. 1954;8(1–6):304–333.
- [12] Park MS, Cho HJ, Kim NK, et al. Ten new recorded species of macrofungi on Ulleung island, Korea. Mycobiology. 2017;45(4):286–296.
- [13] Dai SJ, Vlasák J, Tomšovský M, et al. *Porodaedalea chinensis* (Hymenochaetaceae, Basidiomycota) – a new polypore from China. Mycosphere. 2017;8(6):986–993.
- [14] Ko KS, Jung HS. Three nonorthologous ITS1 types are present in a polypore fungus *Trichaptum abietinum*. Mol Phylogenet Evol. 2002;23(2):112–122.
- [15] Doolotkeldieva T, Bobusheva S. Endophytic fungi diversity of wild terrestrial plants in Kyrgyzstan. Global Adv Res J Microbiol. 2014;3:163–176.
- [16] Prikhodko SL, Fet GN, Umralina AR, et al. Fungi in forest conservation of the Ala-Archa National Park. Institute of Biotechnology for National Academy of Sciences of the Kyrgyz Republic; 2019. Available from: <http://www.plant-biotech.kg/files/att1.pdf>